Human Reproduction vol.11 no.6 pp.1157–1161, 1996

OPINION

The temporary anatomical structures prominent in the first trimester may be fulfilling exchange functions assigned to the placenta in the second and third trimester

S.M.Docherty, R.K.Iles[1], N.Wathen and T.Chard

Departments of Obstetrics, Gynaecology and Reproductive Physiology, St. Bartholomew's Hospital Medical College, West Smithfield, London EC1A 7BE, UK

[1]To whom correspondence should be addressed

The extra-embryonic coelom (EEC) and secondary yolk sac are prominent structures in the gestational sac during the first trimester of human pregnancy, at a time before the definitive placental circulation becomes established. We propose that the EEC and yolk sac play a critical role in the nutrition of early pregnancy, fulfilling exchange functions which are assumed by the placenta at a later stage.

Key words: amniotic fluid/early pregnancy/extra-embryonic coelom/yolk sac

Introduction

The introduction of high-resolution transvaginal ultrasonography has allowed observation and sampling of the embryonic cavities of early gestation (Jauniaux *et al.*, 1991b; Wathen *et al.*, 1991a). This has given new insights into the composition of extra-embryonic coelomic (EEC) fluid and amniotic fluid in the first trimester of pregnancy, and has generated novel hypotheses on the physiology and biochemistry of early gestation.

Formation of the extra-embryonic coelom and yolk sac

After fertilization, a series of rapid mitoses in the zygote forms the morula, which reaches the endometrial cavity 4–5 days later. Accumulation of fluid in intercellular spaces leads to formation of the blastocyst; this comprises an outer layer which will form the trophoblast, an inner cell mass and the blastocoelic fluid, all still encased within the zona pellucida. Implantation commences 7–8 days post-ovulation. The disappearance of the zona pellucida exposes the trophoblast, which adheres to the epithelial cells of the endometrium, penetrates this layer and invades the underlying stroma. This leads to the 'decidual reaction'; the endometrial stroma surrounding the implantation site becomes highly oedematous and vascular, with appearance of large granular lymphocytes.

At the time of implantation the cells of the inner cell mass differentiate to form two layers: a hypoblast layer adjacent to the blastocyst cavity, and an epiblast layer. Together the hypoblast and epiblast form the bilaminar germ disk. The amniotic cavity forms between the epiblast and the trophoblast, while some of the most caudal cells of the epiblast layer proliferate to form a thin membrane which lines the inner surface of the trophoblastic basal lamina and forms the primitive yolk sac. A new population of cells, the extra-embryonic mesoderm, evolves and separates the trophoblast and the yolk sac. Cavities form in this layer which coalesce to form the EEC (Carlson, 1994). The coelom divides the extra-embryonic mesoderm into two layers. Somatic mesoderm lines the trophoblast, and splanchnic mesoderm surrounds the secondary yolk sac and fetus.

Hypoblastic cells migrating from the embryonic disk along the inside of the exocoelomic membrane proliferate and lead to formation of the much smaller secondary yolk sac. During the third week of gestation, gastrulation establishes all three germ layers in the embryo. The primitive streak forms on the surface of the epiblast, with the primitive node at its cephalic end. Epiblast cells invaginate in the region of the streak to form the endoderm and mesoderm. The secondary yolk sac communicates with the ventral aspect of the trilaminar embryo, while the cytotrophoblast-derived amnion meets the ventral aspect of the developing embryo at the site of umbilical cord insertion. As the embryo enlarges, so the amniotic cavity around it expands relative to the EEC. Lateral folding of the embryo during the sixth week of gestation results in narrowing of the base of the yolk sac, with the formation of the yolk stalk. The yolk sac itself becomes more peripheral as gestation progresses. The structures present at around the ninth week of gestation are shown in Figure 1.

Fate of the extra-embryonic coelom and yolk sac

By the 13th week of gestation the amniotic cavity has expanded so that the amnion is in direct contact with the trophoblast; thus the EEC is obliterated. The yolk sac becomes increasingly marginalized and the yolk stalk reduced to a narrow vitelline duct as the amniotic cavity expands. The yolk sac degenerates at around the same time as its stalk becomes covered in amnion and incorporated into the primitive umbilical cord. The connection with the midgut via the vitelline duct is also obliterated at this time (Sadler, 1990).

Possible functions of the extra-embryonic coelom

Recent studies have shown that the composition of the coelomic fluid differs dramatically from that of amniotic fluid, and that there are great variations with gestational age (Jauniaux *et al.*,

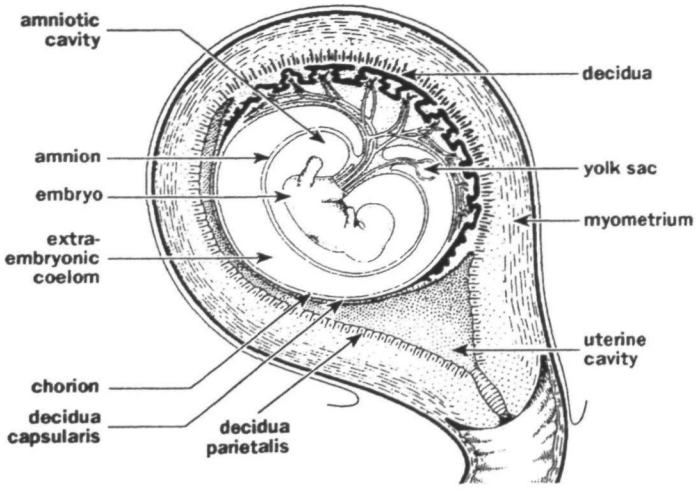

Figure 1. Embryonic cavities at the ninth week of gestation.

1991a,b; Iles *et al.*, 1992; Wathen *et al.*, 1992a). The coelomic fluid contains high concentrations of protein hormones [such as human chorionic gonadotrophin (HCG)], steroids (such as oestradiol and progesterone) and pregnancy-associated compounds [such as insulin-like growth factor binding protein-1 (IGFBP-1) and placental protein-14 (PP14)], whilst the amniotic fluid is virtually devoid of all these materials. Furthermore, fetal waste products such as urea, creatinine and bilirubin are present in high concentrations in coelomic fluid, but not in the amniotic fluid which immediately surrounds the fetus. Alpha fetoprotein (AFP) is the sole exception; it is found in comparable amounts in both cavities (Table I). The different amounts of high molecular weight molecules between the two cavities (despite their intimate contact) might be explained if the amnion is relatively impermeable to large molecules (Jauniaux *et al.*, 1991b). The presence of anionic sites on the basement membrane of the amnion (King, 1985) may result in electrostatic repulsion and steric hindrance of large anionic molecules, and thus represent a significant barrier to permeability (Jones and Jauniaux, 1995). However, a low permeability to anionic, large molecules would not explain the disparity in the amounts of the low molecular weight, lipophilic steroids and vitamins (Campbell *et al.*, 1993; Jauniaux *et al.*, 1993; Campbell *et al.*, 1994; Sourial *et al.*, 1994), nor the equal distribution of AFP. Active transport mechanisms in a dynamic amnion might, therefore, be responsible for initiating and maintaining the disparities. This concept is supported by the presence of high concentrations of bicarbonate in the amniotic fluid and high concentrations of phosphate in the EEC, suggesting the presence of ion anti-ports driving active transport channels. Concentrations of some of these ions in amniotic and coelomic fluids are summarized in Table II. A number of important changes have been observed in the ultrastructure of the amnion during the first trimester. Rich stores of intracellular glycogen are present in the amniotic epithelium during early pregnancy and may represent an energy source in the absence of organelles such as mitochondria or endoplasmic reticulum (Jones and Jauniaux, 1995). The amnion has carbonic anhydrase activity (Benirschke and Kaufmann, 1990). Carbonic anhydrase is an enzyme which catalyses the formation of carbonic acid from carbon dioxide and water, then allowing the dissociation of carbonic acid to give bicarbonate and hydrogen ions. This may imply a role for the amnion in the regulation of pH in amniotic fluid (Jones and Jauniaux, 1995). Similarly, 17β-hydroxysteroid dehydrogenase activity has been reported in the amnion between 7–20 weeks of gestation (Sulcová *et al.*, 1974), which may suggest a role for amnion epithelial cells in the modification of steroids. One possible alternative explanation for the function of the amnion may be as an

Table I. Median value of pregnancy-associated antigens in amniotic fluid, extra-embryonic coelomic (EEC) fluid and maternal serum between 8–12 weeks gestation*

Pregnancy-associated antigen	Reference	Tissue source	Amniotic fluid	EEC fluid	Maternal serum
Alpha fetoprotein (kIU/ml)	Wathen *et al.*, 1991b	Fetal yolk sac	26.0	24.1	6.4
Alpha fetoprotein (kIU/ml)	Jauniaux *et al.*, 1993	Fetal yolk sac	6.1	7.1	–
Cancer antigen 125 (IU/ml)	Campbell *et al.*, 1992a	Fetal	496	35	35
HCG (IU/ml)	Iles *et al.*, 1992	Placental trophoblast	1.73	245	157
HCG (IU/ml)	Jauniaux *et al.*, 1993	Placental trophoblast	1.00	120	81
Total β-subunit HCG (IU/ml)	Iles *et al.*, 1992	Placental trophoblast	6.73	410	141.5
Free α-subunit HCG (μg/ml)	Iles *et al.*, 1992	Placental trophoblast	0.262	17.3	1.3
Progesterone (pmol/ml)	Unpublished data	Placental trophoblast	56.0	850	83.5
Progesterone (pmol/ml)	Jauniaux *et al.*, 1993	Placental trophoblast	23.6	877	69.7
Oestradiol (pmol/ml)	Jauniaux *et al.*, 1993	Placental trophoblast	5083	26978	4448
Unconjugated oestradiol (pmol/ml)	Wathen *et al.*, 1992a	Placental trophoblast	<1.2	2.6	<1.2
Placental protein 14 (ng/ml)	Wathen *et al.*, 1992a	Maternal decidua	77	4416	642
IGFBP-1 (ng/ml)	Wathen *et al.*, 1992b	Fetal/Maternal/Placental	7.5	500	64
Insulin-like growth factor-1 (ng/ml)	Wathen *et al.*, 1992b	Fetal/Maternal/Placental	273	364	98
Human placental lactogen (ng/ml)	Wathen *et al.*, 1992a	Placental syncytiotrophoblast	30.0	80.0	210.0
Prolactin (IU/ml)	Wathen *et al.*, 1993	Maternal decidua	40	371	709
Vitamin A (μmol/l)	Campbell *et al.*, 1994	–	0	0.08	1.85
Vitamin E (μmol/l)	Campbell *et al.*, 1994		0	0.26	17.01
Folate (μg/l)	Campbell *et al.*, 1993	–	2.15	9.9	6.2
Vitamin B12 (ng/l)	Campbell *et al.*, 1993	–	987	3680	405
Cobalamin (ng/l)	Sourial *et al.*, 1994	–	589	3162	427
Pregnancy-associated plasma protein A (mIU/l)	Iles *et al.*, 1994	Placental syncytiotrophoblast	<10	26	1220

HCG = human chorionic gonadotrophin; IGFBP-1 = insulin-like growth factor binding protein-1.
*Modified from Iles *et al.*, 1994, Campbell *et al.*, 1993, 1994.

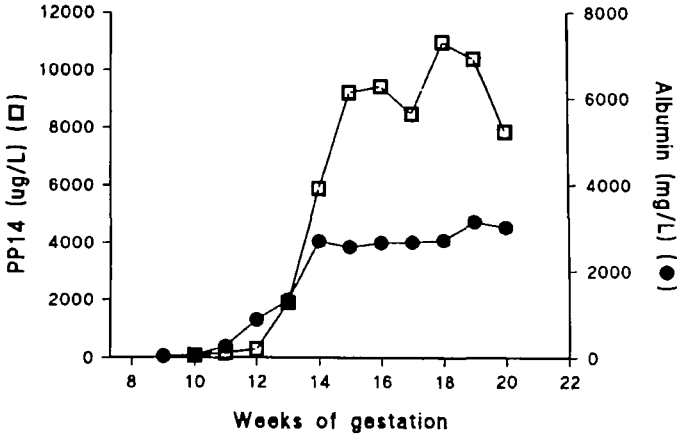

Figure 2. Median concentrations of placental protein-14 (PP14) and albumin in amniotic fluid from 10–20 weeks gestation (taken from Chatzakis *et al.*, 1994).

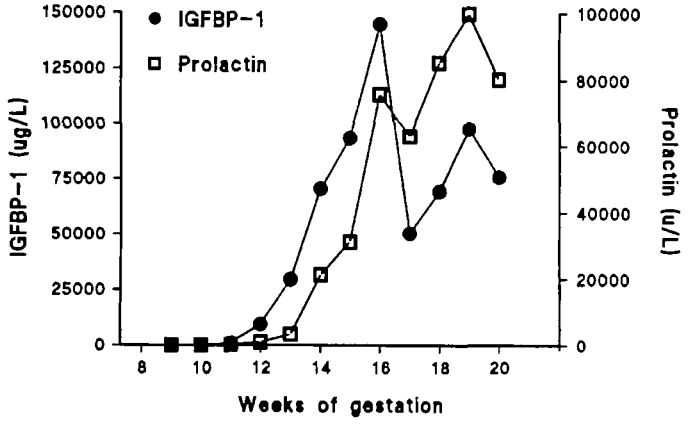

Figure 3. Concentrations of prolactin and insulin-like growth factor binding protein-1 (IGFBP-1) in amniotic fluid from 9–20 weeks gestation (taken from Wathen *et al.*, 1993).

extremely effective barrier against waste products accumulated in the EEC via an as yet unidentified route.

The EEC may segregate highly active molecules including differentiation factors, cytokines, hormones and waste products, thus protecting the poorly keratinized (i.e. highly permeable) fetus at a time when crucial differentiation and organogenesis is occurring. After the obliteration of the EEC at around 12 weeks of gestation, there is a dramatic rise in the concentration of most placental and endometrial proteins in the amniotic fluid (Wathen *et al.*, 1993; Chatzakis *et al.*, 1994; Iles *et al.*, 1994), for example, PP-14 (Figure 2), prolactin and IGFBP-1 (Figure 3). This striking discontinuity at 12–14 weeks is consistent with the loss of transport function by the amnion, since fusion of the amnion and trophoblast would eliminate the reservoir (i.e. the EEC) for export of such proteins. These important changes in transport functions coincide with the appearance of maternal blood flow in the intervillous space (Hustin and Schaaps, 1987).

Removal of waste products from the proximity of the sensitive embryo via the EEC could be an important feature of early gestation. Only later, with the onset of maternal blood flow in the intervillous space, does the placenta subsume this function. Indeed, a state of relative hypoxia is now considered

Table II. Biochemical composition* of amniotic and coelomic fluid as measured at 7–12 weeks gestation in normal pregnancy and analysed using Student's *t*-test

Variable	Amniotic fluid	Coelomic fluid	Significance (*P*)
Sodium (mmol/l)	141.2	138.1	<0.0001
Potassium (mmol/l)	3.98	3.86	0.0038
Chloride (mmol/l)	97.41	110.1	<0.0001
Urea (mmol/l)	3.32	3.9	0.02
Bicarbonate (mmol/l)	34.24	20.9	<0.0001
Creatinine (μmol/l)	37.1	72.31	<0.0001
Calcium (mmol/l)	1.43	2.66	<0.0001
Phosphate (mmol/l)	1.09	2.69	<0.0001
Bilirubin (μmol/l)	0.64	3.4	<0.0001

*From Campbell *et al.*, 1992b.

a prerequisite for induction of placental gene expression (Rodesch *et al.*, 1992). This has been demonstrated for transcription of the gene for vascular endothelial growth factor (VEGF) which is increased in placental fibroblasts grown in hypoxic conditions (Wheeler *et al.*, 1995). The expansion and obliteration of the EEC also coincides with the rise and fall in concentrations of HCG in the first trimester, leading to the suggestion that a tissue closely associated with the EEC (probably the chorion) is the principle source of HCG (Chard *et al.*, 1995). It is notable that concentrations of HCG and its subunits are strikingly higher in the EEC than elsewhere, including maternal blood (Iles *et al.*, 1992; Nagy *et al.*, 1994; Jauniaux *et al.*, 1995).

The EEC may also be involved in the delivery of nutrients to the fetus via the secondary yolk sac and vitelline circulation. Vitamins A, B12, E, folate and cobalamin are present in high concentrations in the EEC (Table I) (Campbell *et al.* 1993, 1994; Sourial *et al.*, 1994).

AFP is unusual in that concentrations are equivalent in both amniotic and exocoelomic cavities; the molecular variants found in both sites show a common derivation from the yolk sac (Jauniaux *et al.*, 1993). AFP is the fetal analogue of albumin (Alpert *et al.*, 1971); there is a 39% sequence homology with the adult molecule (Morinaga *et al.*, 1983), and both are coded on the long arm of chromosome 4. AFP may function similarly to its adult equivalent in showing binding affinities for a variety of ligands. However, AFP differs from albumin in its preferential binding of polyunsaturated fatty acids (reviewed by Deutsch, 1991) and may have a role in transporting these substances to developing cells; AFP could be a transport protein for nutrients and growth factors on both sides of the amnion.

Possible functions of the yolk sac

The secondary yolk sac floats in the EEC, and the cavity is in open connection with the midgut of the developing fetus via the vitelline duct. Its wall comprises an external mesothelial layer of flattened cells (facing the EEC), a vascular mesenchyme, and an endodermal layer of columnar cells facing into the yolk sac cavity (Jauniaux and Moscoso, 1992). It has a well developed microvillous border on its external surface and numerous pinocytotic vesicles, implying an active absorptive

function (Jauniaux *et al.*, 1991a) in addition to its documented biosynthetic functions. The yolk sac produces AFP in its endodermal layer until the 10th week of gestation (Gitlin and Perricelli, 1970). It is probably the major source of protein synthesis (Gulbis *et al.*, 1992) (including albumin and ferritin) at a time prior to embryonic liver maturation. Protein synthesis by the yolk sac ceases after the ninth week of gestation (Jones and Jauniaux, 1995). In addition, the yolk sac synthesizes many enzymes involved in digestion and metabolism, including lactic dehydrogenase, galactosidase, α-glutamyl transferase and acid phosphatase (Buffe *et al.*, 1993). The yolk sac could represent a source of nutrients to the growing embryo at a time when there is relatively poor placental blood supply, and it might also absorb waste products from the EEC, using these in the manufacture of essential molecules. The yolk sac could thus be described as a primitive extra-embryonic 'liver' to the early embryo.

The yolk sac is the first site of haematopoiesis (Moore and Metcalf, 1970), producing nucleated red blood cells in 'blood islands' and seeding the fetal liver and spleen for further haematopoiesis after its decline (reviewed by Tavassoli, 1991), though the precise site of initiation is still unknown. The yolk sac is also proposed as the source of primordial germ cells (Witschi, 1948). Nothing more is known of the functions of the human yolk sac, but the rodent yolk sac is more amenable to investigation; functions include nutritional, endocrine, secretory, excretory, metabolic, haematogenic and immunological (reviewed by Beckman *et al.*, 1990). Furthermore, agents which target the rat yolk sac are highly embryotoxic, emphasizing the overall physiological importance of this structure (Beckman *et al.*, 1990; Brent *et al.*, 1990; Jollie, 1990). In humans, a possible link between a small or absent yolk sac and spontaneous abortion has been suggested (Nogales *et al.*, 1993)

Conclusion

The fact that there are high concentrations of proteins, steroids, and other organic and inorganic molecules in the coelomic fluid but not the amniotic fluid in the first trimester, together with the absence of intervillous blood flow at that time, suggests a pivotal role for the amnion and EEC in segregation and excretion of active molecules and waste products from the developing embryo. The site and prominence of the secondary yolk sac suggest that this structure also plays a crucial role in nutritional, endocrine and metabolic support of the early conceptus.

The disappearance of the EEC and the secondary yolk sac at around 12 weeks of pregnancy coincides with an abrupt increase in amniotic fluid levels of various biological molecules, and the start of perfusion of the intervillous space by maternal blood. This implies that the developed placenta takes over the functions previously fulfilled by the yolk sac, amnion and EEC.

References

Alpert, E., Drysdale, J.W., Schur, P.H. and Isselbacher, K.J.(1971) Human AFP; purification and physical properties. *Fed. Proc.*, **30**, 246.

Beckman, D.A., Koszalka, T.R., Jensen, M. and Brent, R.L. (1990) Experimental manipulation of the rodent visceral yolk sac. *Teratology*, **41**, 395–404.

Benirschke, K. and Kaufmann, P. (1990) *Pathology of the Human Placenta*. 2nd edn. Springer-Verlag, London, UK.

Brent, R.L., Beckman, D.A., Jensen, M. and Koszalka, T.R. (1990) Experimental yolk sac dysfunction as a model for studying nutritional disturbances in the embryo during early organogenesis. *Teratology*, **41**, 405–413.

Buffe, D., Rimbaut, C. and Gaillard, J.A. (1993) Alpha-fetoprotein and other proteins in the human yolk sac. In Nogales, F.F. (ed.) *The Human Yolk Sac and Yolk Sac Tumors*. Springer-Verlag, pp. 109–125.

Carlson, B.M. (1994) *Human Embryology and Developmental Biology*. C.V.Mosby, Baltimore, USA.

Campbell, J., Kitau, M., Cass, P. *et al.* (1992a) CA 125 in matched samples of amniotic fluid, extra-embryonic coelomic fluid and maternal serum in the first trimester of pregnancy. *J. Obstet. Gynaecol.*, **100**, 563–565.

Campbell, J., Wathen, N., Macintosh, M. *et al.* (1992b) Biochemical composition of amniotic fluid and extra-embryonic coelomic fluid in the first trimester of pregnancy. *Brit. J. Obstet. Gynaecol.*, **99**, 563–565.

Campbell, J., Wathen, N., Perry, G. *et al.* (1993) The coelomic cavity: an important site of materno-fetal exchange in the first trimester of pregnancy. *Br. J. Obstet. Gynaecol.*, **100**, 765–767.

Campbell, J., Wathen, N.C., Merryweather, I. *et al.* (1994) Concentrations of vitamins A and E in amniotic fluid, extra-embryonic coelomic fluid, and maternal serum in the first trimester of pregnancy. *Arch. Dis. Child.*, **71**, F49–50.

Chard, T., Iles, R., and Wathen, N. (1995) Why is there a peak of human chorionic gonadotrophin (HCG) in early pregnancy? *Hum. Reprod.*, **10**, 101–104.

Chatzakis, K., Wathen, N., Campbell, J. *et al.* (1994) Dramatic increase in levels of placental protein 14 in amniotic fluid at 10–15 weeks' pregnancy. *Early Hum. Dev.*, **36**, 113.

Deutsch, H.F. (1991) Chemistry and biology of alpha-fetoprotein. *Adv. Cancer Res.*, **56**, 253–312.

Gitlin, D. and Perricelli, A. (1970) Synthesis of serum albumin, prealbumin, alphafetoprotein, alpha 1-antitrypsin and transferrin by the human yolk sac. *Nature*, **228**, 995–997.

Gulbis, B., Jauniaux, E., Jurkovic, D. *et al.* (1992) Determination of protein pattern in embryonic cavities of human early pregnancies: a means to understand materno-embryo exchanges. *Hum. Reprod.*, **7**, 886–889.

Hustin, J. and Schaaps, J.-P. (1987) Echocardiographic and anatomic studies of the maternotrophoblastic border during the first trimester of pregnancy. *Am. J. Obstet. Gynecol.*, **157**, 162–168.

Iles, R.K., Wathen, N.C., Campbell, D.J. and Chard T. (1992) Human chorionic gonadotrophin and subunit composition of maternal serum and coelomic and amniotic fluids in the first trimester of pregnancy. *J. Endocrinol.*, **135**, 563–569.

Iles, R.K., Wathen N.C., Sharma, K.B. *et al.* (1994) Pregnancy-associated plasma protein A levels in maternal serum, extra-embryonic coelomic and amniotic fluids in the first trimester. *Placenta*, **15**, 693–699.

Jauniaux, E., and Moscoso, J.G. (1992) Morphology and significance of the human yolk sac. In *The First Twelve Weeks of Gestation*. Springer-Verlag, London, UK, pp. 192–216.

Jauniaux, E., Jurkovic, D., Henriet, Y. *et al.* (1991a) Development of the secondary human yolk sac: correlation of sonographic and anatomic features. *Hum. Reprod.*, **6**, 1160–1166.

Jauniaux, E., Jurkovic, D., Henriet, Y. *et al.* (1991b) Biochemical composition of exocoelomic fluid in early human pregnancy. *Obstet. Gynecol.*, **78**, 1124–1128.

Jauniaux, E., Gulbis, B., Jurkovic, D. *et al.* (1993) Protein and steroid levels in embryonic cavities in early human pregnancy. *Hum. Reprod.*, **8**, 782–787.

Jauniaux, E., Gulbis, B., Nagy, A.M. *et al.* (1995) Coelomic fluid chorionic gonadotrophin and protein concentrations in normal and complicated first trimester human pregnancies. *Hum. Reprod.*, **10**, 214–220.

Jollie, W.P. (1990) Development, morphology, and function of the yolk-sac placenta of laboratory rodents. *Teratology*, **41**, 361–381.

Jones, C.J.P. and Jauniaux, E. (1995) Ultrastructure of the materno-embryonic interface in the first trimester of pregnancy. *Micron*, **26**, 145–173.

King, B.F. (1985) Distribution and characterisation of anionic sites in the basal lamina of developing human amniotic epithelium. *Anat. Rec.*, **212**, 57–62.

Moore, M. and Metcalf, D. (1970) Ontogeny of the haematopoietic system: yolk sac origin of *in vivo* and *in vitro* colony forming cells in the developing mouse embryo. *Br. J. Haematol.*, **18**, 279.

Morinaga, T., Sakai, M., Wegmann, T.G. and Tamaoki, T. (1983) Primary structures of the human alpha-fetoprotein and its mRNA (cDNA clones/the domain structures/molecular evolution). *Proc. Natl. Acad. Sci. USA*, **80**, 4604–4608.

Nagy, A.M, Jauniaux E., Jurkovic, D. and Meuris, S. (1994) Placental production of human chorionic gonadotrophin I and J subunits in early pregnancy as evidenced in fluid from the exocoelomic cavity. *J. Endocrinol.*, **142**, 511–516.

Nogales, F.F., Beltran, E. and Gonzalez, F. (1993) Morphological changes of the secondary human yolk sac in early pregnancy wastage. In Nogales, F.F. (ed.) *Human Yolk Sac and Yolk Sac Tumors*. Springer-Verlag, pp. 174–194.

Rodesch, F., Simon, P., Donner, C. and Jauniaux, E. (1992) Oxygen measurements in endometrial and trophoblastic tissues in early pregnancy. *Obstet. Gynecol.*, **80**, 283–285.

Sadler, T.W. (1990) *Langman's Medical Embryology*. 6th edn., Williams and Wilkins, Edinburgh, UK.

Sourial, N.A., Campbell, J., Wathen, N. *et al.* (1994) What is the role of transcobalamins? *Advances in Thomas Addison's Diseases*. Vol. 2. J. Endocrinol. Ltd, Bristol, pp. 241–245.

Sulcová, J., Jirásek, J.E. and Stárka, L. (1974) 17J-hydroxysteroid dehydrogenase activity in human amniotic epithelium. *Endokrinologie*, **63**, 249–253.

Tavassoli, M. (1991) Embryonic and fetal hemopoiesis: an overview. *Blood Cells*, **17**, 282–286.

Wathen, N.C., Cass, P.L., Campbell, D.J. *et al.* (1991a) Early amniocentesis: alphafetoprotein levels in amniotic fluid, extaembryonic fluid and maternal serum between 8 and 13 weeks. *Br. J. Obstet. Gynaecol.*, **98**, 866–870.

Wathen, N.C., Cass, P.L., Kitau, M.J. and Chard, T. (1991b) Human chorionic gonadotrophin and alphafetoprotein levels in matched samples of amniotic fluid, extra-embryonic coelomic fluid and maternal serum in the first trimester of pregnancy. *Prenatal Diagn.*, **11**, 145–151.

Wathen, N.C., Cass, P.L., Campbell, D.C. *et al.* (1992a) Levels of placental protein 14, human placental lactogen and unconjugated oestriol in extra-embryonic coelomic fluid. *Placenta*, **13**, 195–197.

Wathen, N.C., Wang, H.S., Cass, P.L. *et al.* (1992b) Insulin-like growth factor-1 and insulin-like growth factor binding protein-1 in early pregnancy. *Early Hum. Dev.*, **28**, 105–110.

Wathen, N.C., Campbell, D.J., Patel, B. *et al.* (1993) Dynamics of prolactin in amniotic fluid and extra-embryonic coelomic fluid in early pregnancy. *Early Hum. Dev.*, **35**, 167–172.

Wheeler, T., Elcock, C.L. and Anthony, F.W. (1995) Angiogenesis and the placental environment. *Placenta*, **16**, 289–296.

Witschi, E. (1948) Migration of the germ cells of human embryos from the yolk sac to the primitive gonadal folds. *Contr. Embryol. Carnegie Inst.*, **32**, 67–80.

Received on December 28, 1995; accepted on March 21, 1996